INSTRUCTIONS FOR COMPLETING THE NIH INTRAMURAL ANIMAL STUDY PROPOSAL FORM (4/03) - excerpt

SECTION E: RATIONALE FOR ANIMAL USE

3) Number of animals:

Most studies at NIH are either new experimental paradigms or pilot studies, and therefore, applying statistical principles to justify numbers can be difficult due to the lack of knowledge regarding the potential outcomes and variance. Under these circumstances, it is still important to show how animal numbers were generated. It is also reasonable to ask for larger group sizes (i.e. 10/group versus 6/group) with the caveat that early statistical analysis will be applied and the group size decreased if the statistics support using smaller groups.

For Example: We estimate that 200 animals will be necessary for this study because we will be using five (5) animals per group, and examining the effects of five (5) compounds (including vehicle), at four (4) doses of drug per compound, with two (2) replications, (to assure reproducibility), per determination. Therefore the total numbers requested will be: 5 x 5 x 4 x 2 = 200.

In studies that are a continuation of ongoing work or that parallel current or previous experiments, if the degree of variance is known, then a short summary of statistical principles that were applied to arrive at your group size(s) is appropriate (i.e. power, standard deviations, etc.). If applying statistical principles is not directly applicable, then a short summary of reasoning applied to arrive at group sizes should be provided.

When the use of animals is for the **harvesting of normal tissues, organs or fluids for in vitro use:** Briefly cite expected usage levels to provide the quantity of tissues or fluids needed for the study. If no prior experience is available, state an anticipated tissue/fluid harvest per animal with a description of the process. These numbers should be scientifically justified and not based solely on personnel availability.

For Example: In our experience, 10 rats are required to generate enough cells for one experiment. Since we can conduct one experiment per week we need 520 rats per year. This number of animals and rate of use will enable us to test 10 drugs at the desired dosage.

If rodents are to be used for breeding, then the following categories can be used in justifying/clarifying your animal numbers: 1) numbers used for breeding (including founders, background strain, and retained progeny), 2) total numbers of expected progeny, 3) the numbers of progeny intended for experimental use or export, 4) the numbers of progeny needed for continuation of the experimental line, and 5) the numbers that will be euthanized due to undesirable genotype. Animals with undesirable genotype are not counted into animal total numbers if they are euthanized before weaning, but *for completeness* should be listed in the numerical breakdown. In

addition, it is helpful to provide the objectives for the breeding (i.e. homozygous pups for experiments, or back crossing to establish a genetic mutant on a homogeneous background). Enough detail of the breeding schemes should be included to allow the ACUC to assess the appropriateness of requested animal numbers. An outline, a table, or a flow chart for each strain listed can be very helpful in presenting this information. This breakdown of breeding categories should be included under Section F as part of the experimental design and referenced in Section E.3. See **Attachment 1** (below) for examples of commonly employed breeding schemes and instructions on estimating animal numbers for this purpose.

ATTACHMENT 1

Examples of Breeding Schemes

Glossary
Mutant = knock-out or transgenic mouse.
Wild type (+/+) **=** pups from mutant crosses that do not carry the gene of interest at either allele, or background strain that does not carry a mutation.
Heterozygous (het, Aa, or +/-) **=** the mutant gene of interest is carried as one dominant allele and one recessive allele.
Homozygous (hom, AA, or aa) **=** the mutant gene of interest is carried as either dominant or recessive at both alleles.
Background strain = the inbred strain or strains on which the mutant gene was established, e.g. C57BL/6 or 129.
Congenic strains = two strains that are genetically identical except for a short chromosomal segment, achieved by backcrossing to an inbred strain (usually 10 backcrosses).

Maintaining an Established Mouse Strain/Line

When predicting the number of mice required in maintaining an established strain, actual breeding data is used or certain assumptions are made as follows:

- Age of new breeders - both male and female usually mature at 2 months of age.
- Number of pups per litter - 5 pups/litter if average litter size is unknown.
- Number of litters per female - female will produce 4 litters with no postpartum breeding and is typically retired after 6 months.

Example: 2 breeder pairs X 4 litters/each X 5 pups/litter = 40 pups

Mice needed for general experiments

After predicting the number of pups that a breeding pair will produce, it will be necessary to determine how many of the pups will carry the gene of interest and then work backwards to determine the number of breeding pairs needed to support the research. The number of mice needed for an experiment may depend on the phenotype, background strain variability, etc., that is known or thought to occur in the mice. Therefore, it may be useful to include a percentage of all pups, rather than only homozygous mutants (-/-) in the experimental design.

- Recessive or Dominant Mutant (a/a or A/A) maintained via het X het breeding
  Y = number of mutant mice / experiment
  25% of pups will carry gene of interest as (-/-)
  Y/0.25 = total pups needed

  Example:
  Let Y = 100
  100 / 0.25 = 400 pups
  Assume 5 pups/litter X 8 litters/yr = 40 pups/pair/yr
  Therefore: 400 pups/40 = 10 breeder pairs/yr (or 20 breeders total)
  Total mice: 400 pups + 20 breeders = 420 mice

- Recessive or Dominant Mutant (a/a or A/A) maintained via het X hom breeding
  Y = number of mutant mice / experiment
  50% of pups will carry gene of interest as (A/- or a/-)
  Y/0.50 = total pups needed

  Example: Let Y=100
  100 / 0.5 = 200 pups

Assume 5 pups/litter X 8 litters/yr = 40 pups/pair/yr
Therefore: 200 pups/40 = 5 breeder pairs/yr (or 10 breeders total)
Total mice: 200 pups + 10 breeders = 210 mice

Mice needed to generate a congenic line (homogeneous background)

When establishing a new mutant model, it is usually desirable to maintain it on a homogeneous background strain. Many mutants are created on a mixed strain background (usually B6 and 129) that ultimately can interfere with interpretation of experimental results. If the mutant is on a mixed background, a series of backcrosses will both stabilize the allelic position of the mutation (if transgenic) and create a homogeneous background strain. Ideally 10 backcrosses should be performed, which will achieve 99.8% homogeneity. At a minimum, 4 backcrosses should be performed to achieve 94% homogeneity.

Assumption: will maintain two backcross breeding pairs per generation, will produce 5 pups per breeder pair or 10 pups total per generation, and will screen prior to weaning (*if not, then must count +/+ pups in total*).

Breeding scheme:

Donor het x background strain (+/+)	= N1
N1 het X (+/+)	= N2
N2 het X (+/+)	= N3
And so on until N10	

Example:
50% of pups will carry gene of interest as het (+/-)
10 pups X 0.5 = 5 mice / generation
5 mice/generation x 10 generations = **50** donor mice

Need two background strain (+/+) mice / generation
2 x 10 generations = **20** background mice
(Also **2** original donor mice)

Total = 50 + 20 + 2 = 72 mice
Total if screen after weaning: 50 + 20 + 2 + 50(+/+ pups) = 122 mice

Total mice accountability

In addition to the total number of mice needed for an experiment, there must be accountability for the following mice:

- Breeding mice B when mice are bred to maintain a line or produce animals for experiments, some will need to be held back to replenish the breeding pool.
- Cull mice B if pups are genetically screened and culled prior to weaning, they DO NOT have to be counted towards the total number. If genetic screening is performed at or after weaning, ALL MUST be counted toward the total number listed on the protocol.
- Remember, female breeders are usually retired after 6 months.

Example: 2 heterozygous breeder pairs X 4 litters X 5 pups = 40 pups +
4 breeders = 44 mice;

- 10 (-/-) are desired mutants;
- 8 (+/+) used for negative control;
- 2 (+/-) females used to replenish breeding stock at 6 months; and
- 2 (+/+) and 18 (+/-) are euthanized.